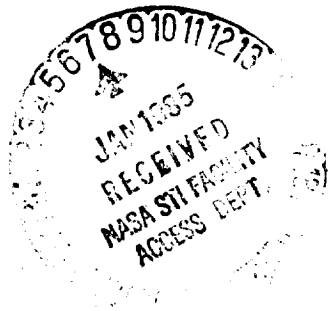

National Aeronautics and Space Administration

EARTH RESOURCES LABORATORY

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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
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NSTL STATION, MISSISSIPPI 39529

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ABSTRACT

A promising scheme for constructing a versatile closed bioregenerating life support system using C_3 and C_4 type plants has been demonstrated. Research at NASA's National Space Technology Laboratories during the past 6 years has demonstrated the potential of higher plants in balancing and stabilizing a bioregenerative life support system. This research demonstrated that: (a) higher plants such as water hyacinth, duckweed, cattail and cherry tomatoes can utilize human waste as a complete growth media, (b) conventional food plants such as tomatoes, sorghum, corn, potatoes, cucumbers and squash can utilize decomposing water hyacinth and duckweed as a substrate for supplying all of their nutritional growth requirements, (c) the biological decomposition rate of water hyacinths approaches or equals its growth rate when the harvested plants are used as a substrate for growing other plants, (d) water hyacinth and duckweed have the potential of being used directly or indirectly for supplying man's food requirements while revitalizing air, (e) juices from water hyacinth can be used to produce high quality protein in the form of yeast cells, (f) water hyacinth can absorb and metabolize organics directly from wastewaters acting in a chemical filtration capacity, and (g) a combination of water hyacinth, duckweed and cattail is capable of maintaining growth under varied light and temperature fluctuations.

INTRODUCTION

In pursuance of U.S. goals in space exploration, astronauts and scientists may need to stay for extended periods of time in celestial stations and planetary bases. As the number of people, length of time, and remoteness in space increases, the total reliance on expendables for air, water, and food becomes more and more impractical. Therefore, a closed or partially closed ecological life support system that is reliable, stable, and reproducible needs to be developed to regenerate the waste into food, oxygen, and potable water.

The first attempts to solve this problem employed the use of physico-chemical methods. The primary physico-chemical methods in consideration are wet oxidation, incineration, pyrolysis, and steam reformation. All of these systems produce stabilized inorganic products. Although extensive research has been done on these systems, many questions still need to be addressed, particularly as to whether these minerals can be readily solubilized and returned to the food production system in a state compatible with biological systems.

Over the past 25 years, limited research in the U.S. has been directed toward the development of bioregenerative life support systems (1-6). These systems are usually challenged with questions of reliability, stability, and efficiency. Bioregenerative systems would require more space for waste stabilization than the physico-chemical systems, but offer much greater potential in waste recycling, food production, etc. The development of such a system is a biological and bioengineering challenge that would have many earthly applications in addition to future space station needs.

Most of the previous research in the U.S. has concentrated on the utilization of algae (chlorella) with occasional studies on higher plants and chemosynthetic bacteria (1-7). In Russia, however, methods using higher plants as producers of oxygen, food, and water and as consumers of carbon dioxide have been investigated extensively (8,9).

NASA has conducted research during the past 6 years at the National Space Technology Laboratories (NSTL) in Mississippi using vascular aquatic plants for

treating domestic sewage and recycling the elements from human waste into high quality protein, vitamins, minerals, animal feed, fertilizer, energy, and purified water (14-21). This research has been directed toward solving wastewater treatment problems at NSTL and developing technology for using higher plants in waste recycling systems for future space applications. This paper describes how this technology is applicable to closed life support systems.

Three vascular aquatic plants, water hyacinths (Eichhornia crassipes), cattails (Typha spp.) and duckweeds (Limna spp., Spirodela spp. and Wolffia spp.) and two terrestrial plants, cherry tomatoes (Lycopersicon spp.) and grain sorghum (Sorghum sudanese) were chosen as five of the more promising plants that could possibly be used in forming the nucleus of a closed life support system where human waste is recycled through higher plants to produce man's requirements in oxygen production, carbon dioxide removal, sodium chloride recycling, water purification, and food in the form of high quality protein, carbohydrates, vitamins, and minerals. Numerous other plants are also candidates for closed ecological systems and additional research could change the nature of plants used in such a proposed system.

Water hyacinths were chosen as a candidate for the C_3 module in Figure 1 because of the ability of this plant to: (a) grow profusely using raw sewage as a substrate, (b) reproduce by vegetative off-shoots, (c) maintain a dynamic growth rate for maximum oxygen production, (d) concentrate sodium chloride in plant tissue (2-4% dry wt.), (e) act as a complete growth substrate, during decomposition of harvested plants, for growing conventional food plants, (f) produce high concentrations of vitamins, minerals and proteins which can be extracted from the plants as food, and (g) produce high quality single cell protein from plant juices.

Duckweeds were chosen because they demonstrate similar growth and nutritional characteristics as water hyacinths in addition to being more cold tolerant and requiring less light for growth. Duckweeds are small floating plants that normally grow beneath the water hyacinth leaves until frost or other processes kill the hyacinth leaves; then the duckweeds become the dominant plant. A combination water hyacinth-duckweed wastewater treatment system allows for maximum surface coverage with increased photosynthesis, therefore increased waste treatment and oxygen production per surface area.

Cattails were added as a candidate for additional redundancy because of their increased salt and cold tolerance and high productivity. They have also been used as human food by various peoples throughout the world (33).

Cherry tomatoes were chosen because they are: (a) among the easiest of the vegetable plants to grow and maintain, (b) can be grown in raw sewage, decomposing water hyacinth and dilute urine solutions, and (c) their seeds remain viable after cycling through man's digestive track and biological wastewater treatment systems.

Grain sorghum is a promising candidate for the C_4 module because of its high photosynthetic rate which should add additional stability to the phytotron system while producing grain for bread and other food products.

Information on C_3 and C_4 photosynthetic pathways are given to help the reader understand the rationale for proposing that separate C_3 and C_4 modules be incorporated into a life support phytotron system. Extensive data is given on water hyacinths with limited data on the other plants.

1. C_3 and C_4 Photosynthesis Pathways

It was assumed until the mid-1960's that the mechanism of photosynthesis was basically the same in all plants. The three-carbon (C_3) pathway where the carbon dioxide that the leaf has admitted from the surrounding air reacts with Ribulose Diphosphate (RuDP) in a reaction catalized by the enzyme DuDP carboxylase forming two molecules of phosphoglyceric Acid (PGA) with three carbon atoms each. In subsequent reactions some of the PGA is converted to end products (Carbohydrates, Amino acids, etc.) of photosynthesis, and some of it utilized to regenerate molecules of RuDP so that they can again serve as acceptors of carbon dioxide. The loop thus closed makes the process of fixing carbon dioxide a self-sustaining cycle driven by energy derived from light. The C_3 or Calvin-Benson pathway is utilized by the majority of plants on earth.

In the Mid-1960's, however, researchers discovered that certain plants such as sugar cane, sorghum, corn and many others utilize a C_4 or Hatch-Slack pathway. Four-carbon photosynthetic pathway occurs in some specially adapted plants. Carbon dioxide entering the leaf reacts with phospho-enol-pyruvate (PEP), a three-carbon compound, to form four-carbon Oxaloacetic Acid (OAA) from which Malic Acid and Aspartic Acid are formed. The acids are transported from the mesophyll cells,

which are in the outer part of the leaf, to the inner bundle-sheath cells. There carbon dioxide is released from the four-carbon compounds and pyruvic acid, a three-carbon compound, is formed. The carbon dioxide is now fixed again in the usual cycle. The pyruvic acid returns to the mesophyll cells where it acquires a phosphate group from Adenosine Triphosphate (ATP) to form PEP, thus regenerating the initial carbon dioxide acceptor molecules. This additional cycle for fixing carbon dioxide helps to increase the overall efficiency of utilizing carbon dioxide. At the chemical level, the C_4 pathway does not differ completely from the C_3 pathway; instead, the former pathway involves an extra series of steps in the initial capture of carbon dioxide.

The major differences between C_3 and C_4 plants are that C_4 plants are generally more productive, transpire less water, are capable of photosynthesizing at higher temperatures, are capable of photosynthesizing even in extremely carbon dioxide limited atmospheres and are not inhibited by the presence of oxygen.

The inhibitory effect of oxygen on the photosynthetic rate of C_3 plants is largely due to the fact that oxygen increases the rate of photorespiration. Carbon dioxide fixed during photosynthesis is released during respiration, resulting in a lower net amount of carbon fixed. It has been estimated that at atmospheric oxygen concentrations C_3 plants respire approximately 50% of the carbon they fix during photosynthesis. Since the leaf anatomy of C_4 plants allows them to recapture respired carbon dioxide, this loss is not observed in C_4 plants. With a reduction of the atmospheric oxygen concentration, respiration is greatly reduced, and C_3 plants become as efficient at photosynthesis as C_4 plants. A second reason for oxygen's inhibitory effect on the photosynthetic rate of C_3 plants is that DuDP carboxylase is inhibited by oxygen, whereas PEP carboxylase is not. Another way of increasing photosynthetic rates of C_3 plants is by increasing the atmospheric concentration of carbon dioxide. This allows the less efficient DuDP carboxylase to become saturated (negates carbon dioxide loss) and steps up production formation (29).

Since the photosynthetic rates of C_3 plants are increased with high carbon dioxide and low oxygen levels, air from the living area of a space station should first enter the C_3 module. The lower carbon dioxide and higher oxygen levels from the C_3 module can then flow through the C_4 module back to the living area without suppressing significantly the photosynthetic rate of C_4 plants and can possibly be used to maintain an oxygen reserve higher than 21%.

II. Biological Waste Treatment and Food Production for Closed Life Support System

The concept of growing vascular aquatic plants directly on human waste for wastewater treatment has been demonstrated extensively during the past several years (22-28). This concept has produced valuable results which can possibly be used in developing a bioregenerative life support phytotron for closed ecological systems.

Such a bioregenerative life support phytotron as shown in figure 1 would be divided into two separated modules. The major module would sustain C_3 plants. The smaller module would sustain C_4 plants which can utilize CO_2 in much lower concentrations and tolerates higher oxygen levels than C_3 plants (29).

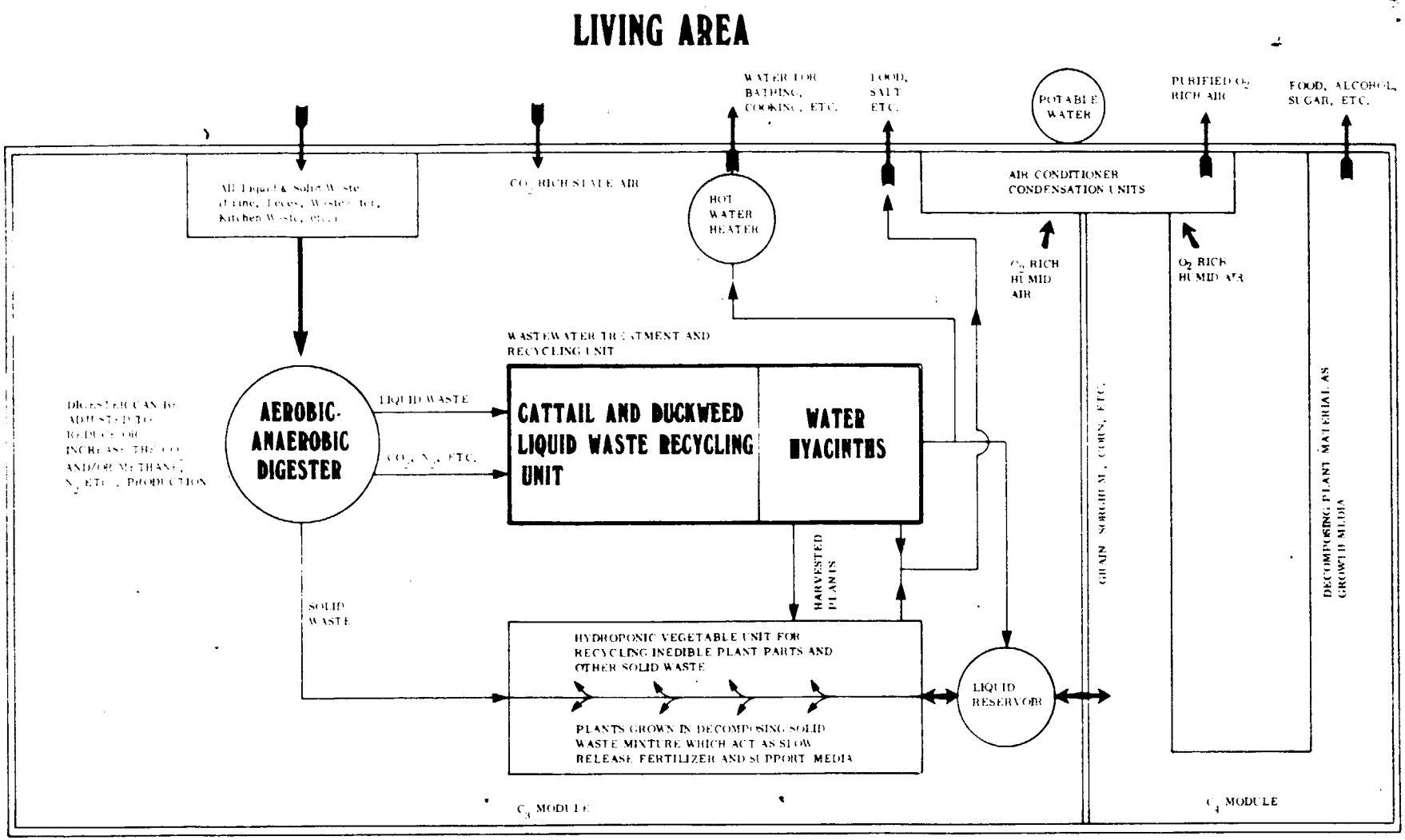
All waste will be pumped into an anaerobic/aerobic digester in the C_3 module where solid settling and partial microbial digestion will be accomplished. After several hours the liquid portion will be pumped into an aquacultural chamber containing cattails, duckweeds and water hyacinths. As an option this chamber could contain cherry tomatoes, and the solids could be pumped onto growth chambers containing inedible plant material. The aquatic plants or tomatoes will continue treating the wastewater, which will then exit routed to one of two places: (1) the composting chamber to water food plants growing there and/or (2) the hot water heater where sterilization removes any pathogenic microorganisms. This water will be used for non-potable water. Drinking water will be obtained by condensing the large quantities of water vapor pumped into the air by the transpiring plants in both C_4 and C_3 modules.

The C_4 module will be used to grow grain sorghum (Sorghum sudanese) and possibly corn (Zea mays) in which the grain will be used for bread and the stalk juices used for syrup, sugar, alcohol, etc. The inedible parts will be recycled back through the growth chambers along with liquids and other plant material from the C_3 module. Air exchange will be directly with the living area or flow from the C_3 to the C_4 chamber because the higher oxygen content in this chamber could reduce photosynthetic rates if vented into the C_3 module.

III. Water Hyacinth, Cattails and Cherry Tomatoes
For Treating Human Waste

To simulate the aerobic/anaerobic digester outlined in the Life Support Phytotron Unit Concept shown in Figure 1, experiments were conducted using water hyacinths and cattails. A 111 liter plastic container was filled with raw sewage collected from the influent to NSTL sewage Lagoon #1. This sewage was allowed to stand for 24 hours and 85 liters pumped into water hyacinth and cattail metal troughs (Figures 2 and 3). The troughs used for these experiments were approximately 0.6 m wide x 3.0 m long x 0.3 m deep. The water hyacinth trough contained sewage from the 24 hour digester and water hyacinths while the cattail trough was filled to a depth of 18 cm with gravel in which the cattails were planted. The bottom 15 cm depth contained 2.5-5.0 cm diameter rocks with the top 3.0 cm containing small pea gravel. Samples of experimental data from these experiments are given in Tables 1, 2, and 3 (36).

A more conventional hydroponic system was used for treating raw sewage with cherry tomatoes. Twelve 20 cm diameter plastic pots filled with oyster shells containing a 3 cm surface layer of small pea gravel were placed in holes cut out in a 20 cm diameter plastic pipe which drained into a reservoir containing 40 liters of raw sewage (Figure 2). The evapotransporation loss was adjusted with distilled water before analysis and new sewage added every 7 days. Raw sewage was pumped through the tomato roots for 15 minutes every hour on a 24 hour cycle. Table 4 shows a set of data covering a 21 day water quality monitoring period from this experiment.



LIFE SUPPORT PHYTOTRON UNIT

Figure 1. Life Support Phytotron Unit

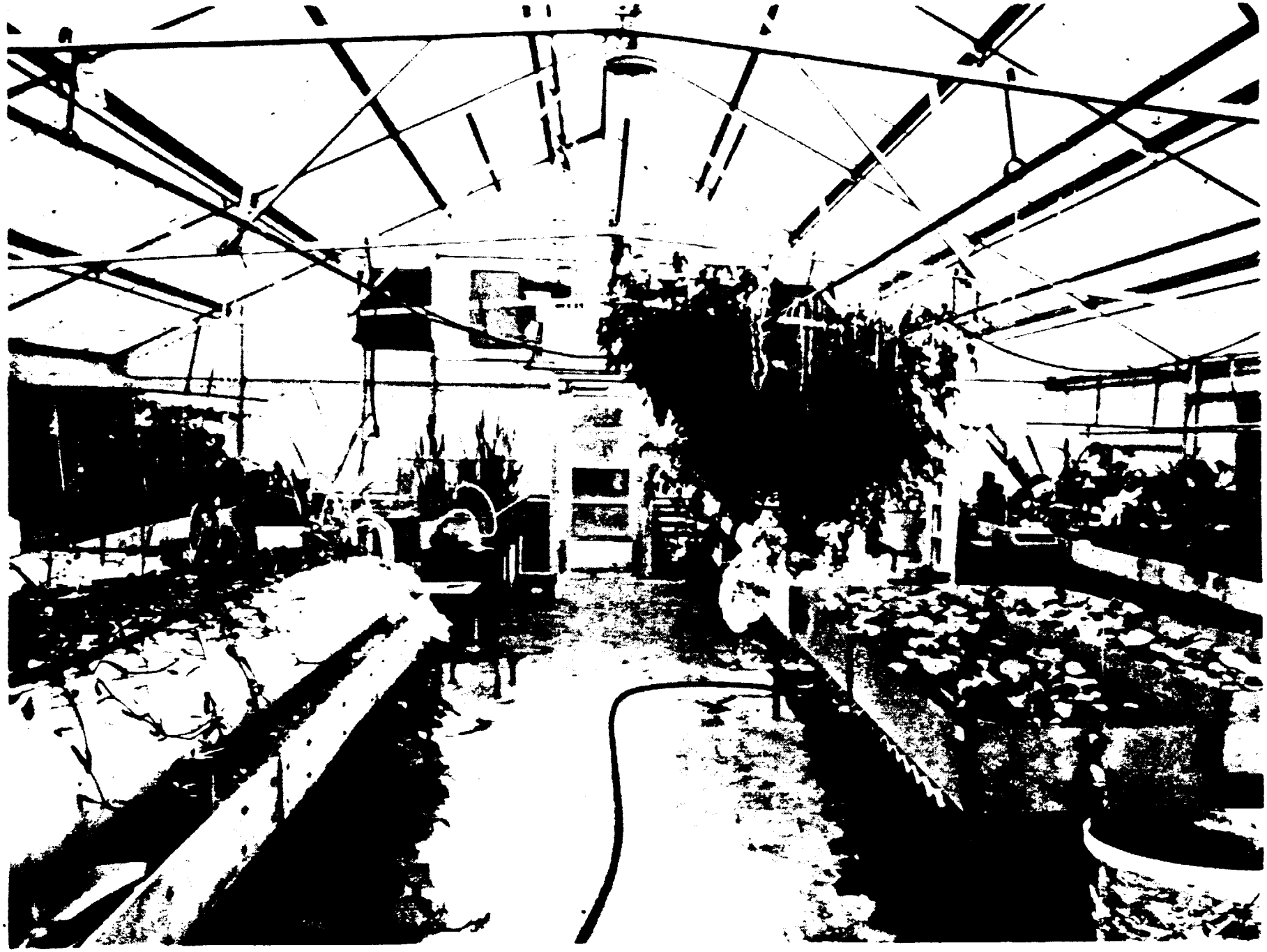


Figure 2. Greenhouse Experiments Growing Water Hyacinths and Other Vascular Plants in Sewage.

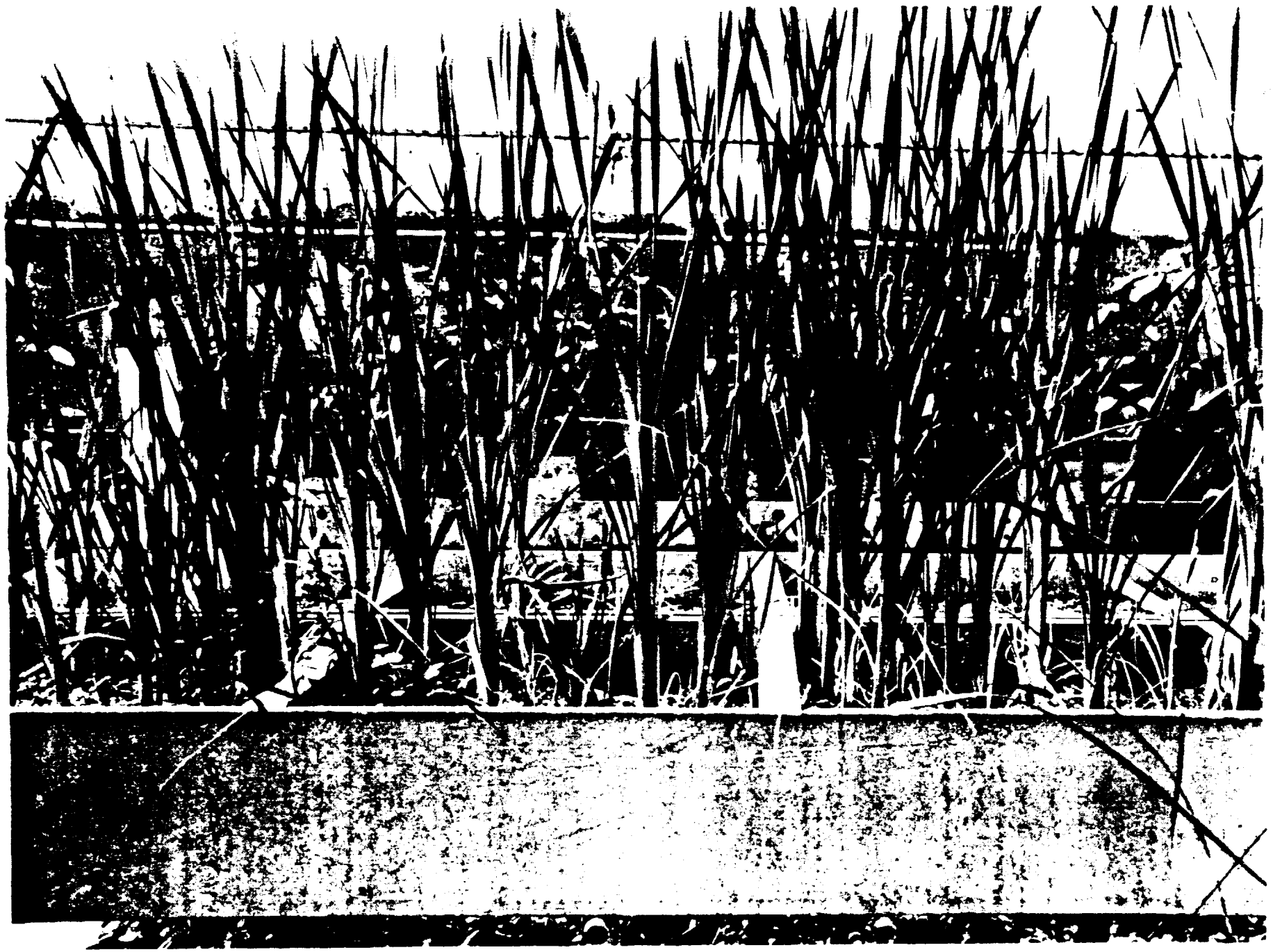


Figure 3. Cattails Growing in Sewage.

Table 1. Data Before and After Treating Raw Sewage 24 Hours in a Closed Settling Tank.

Experiments	Temp. C°		BOD_5, mg/ℓ		TSS, mg/ℓ		TKN, mg/ℓ		TP, mg/ℓ		NH_3, mg/ℓ	
24-hour Settling Tank	Daily Ave. Min.	Max.	In	Out	In	Out	In	Out	In	Out	In	Out
1	18	39	175	88	127	55	21	19	4.4	3.8	11	18
2	16	33	155	48	221	29	19	17	3.6	2.9	18	16
3	20	34	120	54	76	24	24	24	3.9	2.7	17	17
Average	18	35.3	150	60	141	36	21.3	20	3.9	3.1	15.3	17

BOD_5 = Five day Biochemical Oxygen Demand

TSS = Total Suspended Solids

TKN = Total Kjeldahl Nitrogen

TP = Total Phosphorus

NH_3 = Ammonia

Table 2. Effluent From 24 Hour Raw Sewage Settling Tank Treated 48 Hours With Water Hyacinths.

Experiments	Temp. C°		BOD_5, mg/ℓ		TSS, mg/ℓ		TKN, mg/ℓ		TP, mg/ℓ		NH_3, mg/ℓ	
Water Hyacinth Trough	Daily Ave. Min.	Max.	In	Out	In	Out	In	Out	In	Out	In	Out
1	18	35	42	2	18	8	14	3.3	3.0	0.7	25	1.5
2	19	39	62	3	6	0	25	5.6	3.6	0.9	18	4.3
3	20	39	56	2	29	15	21	7.7	4.1	1.7	7.4	3.4
Average	19	37.7	53.3	2.3	17.7	7.7	20	5.5	3.6	1.1	16.8	3.1

Table 3. Effluent From 24 Hour Raw Sewage Settling Tank Treated 48 Hours With Cattails.

Experiments	Temp. C°		BOD_5, mg/ℓ		TSS, mg/ℓ		TKN, mg/ℓ		TP, mg/ℓ		NH_3, mg/ℓ	
Cattail-Rock Filled Trough	Daily Ave. Min.	Max.	In	Out	In	Out	In	Out	In	Out	In	Out
1	21	34	76	1	53	10	17	3	3.6	1.4	15	2
2	19	39	64	5	30	2	14	6	3.1	1.2	11	4
3	15	39	60	8	30	26	18	5	3.3	0.8	18	0.8
Average	18.3	37.3	66.7	4.7	37.7	12.6	16.3	4.7	3.3	1.1	14.7	2.3

Table 4. Raw Sewage Treated Seven Days With Cherry Tomatoes.

Experiments	Temp. C°		BOD_5, mg/ℓ		TSS, mg/ℓ		TKN, mg/ℓ		TP, mg/ℓ		NH_3, mg/ℓ	
Hydroponic System	Daily Ave. Min.	Max.	I	F	I	F	I	F	I	F	I	F
1	18	37	140	2	39	7	19.1	2.1	2.9	0.65	14.5	1.1
2	17	37	350	4	79	0	24.4	6.1	4.8	1.50	15.0	<0.10
3	20	37	120	1	53	0	28.9	4.5	4.0	0.75	23.8	<0.10
Average	18.3	37	203	2.3	57	2.3	24.1	4.2	3.9	0.97	17.8	<0.40

I = Initial

F = Final

IV. Composition of Water Hyacinths, Duckweeds and Cattails Grown on Human Waste

The nutritional composition of duckweeds grown in the NSTL sewage lagoons and artesian well water at NSTL is compared with water hyacinths, water hyacinth extracts, yeast cells, and cattails in Tables 5, 6, 7 and 8. The amino acid composition of soybean meal, a major food source, is compared with the non-conventional aquatic plant proteins in Table 6. Although duckweeds and cattails are not common food plants, they have been used in various parts of the world for food. One species of duckweed, Wolffia, has been used as a vegetable by the Burmese, Laotians and the people of Northern Thailand for many generations (30). Other species of duckweeds have been used as animal feed (31-32) and there is no reason known to this author why these species cannot be used as human food. Of all wild plants, cattails have been called the most useful emergency food source, and traditionally they have been important foods to native peoples throughout the world. These plants are found in fresh and brackish shallow waters all over the world. In time of food scarcity, several nations have considered cattail rhizomes a source of starch and the seeds a source of edible oil. Young cattail stem can be eaten in salads or as a green vegetable (33).

Table 5. Percent Composition of Dry Duckweeds, Cattails, Water Hyacinth Leaf Protein Extract, Water Hyacinth Leaves and Yeast Cells.

Material	Crude Protein	Fat	Crude Fiber	Ash	Total Carbohydrate*
Duckweed no. 1	25	3.05	11.8	12.0	59.5
Duckweed no. 2	39.7	3.40	15.6	12.5	44.4
Water hyacinth leaves	31.3	2.30	13.7	11.7	54.7
Leaf protein extract	57.8	3.54	2.16	4.7	33.6
Single Cell protein Candida tropicalis	48.6	0.57	2.32	3.37	47.5
Cattails tops	14.0	1.35	27.5	11.3	73.4

*Total carbohydrate content was calculated as 100% - (% crude protein + % fat + % ash).

Table 6. Amino Acid Composition of Soybean Meal as Compared to Yeast Grown on Juices From Pressed Water Hyacinths, Protein Extract From Water Hyacinth Leaves, Dried Water Hyacinth Leaves and Duckweeds Grown in Low and High Nutrient Solutions.

g/100 g Crude Protein

Amino Acids	Soybean Meal	Cattail Tops*	Single Cell Protein*** Candida tropicalis	Water Hyacinth Leaf Extract	Duckweed No. 1**	Duckweed No. 2*	Water Hyacinth Leaves*
Lysine	6.49	4.50	8.86	6.80	5.53	6.44	5.96
Histidine	2.63	2.00	1.03	2.38	1.69	2.05	2.31
Arginine	6.98	5.20	4.24	6.89	6.82	7.99	5.49
Aspastic	12.18	11.50	11.61	9.31	14.72	9.68	12.63
Threonine	4.26	5.20	5.73	4.99	4.46	4.61	4.56
Serine	5.51	5.40	4.90	4.60	4.68	4.68	4.29
Glutamic	19.36	12.90	12.02	12.14	13.14	12.67	11.56
Proline	5.29	3.80	4.09	5.28	4.57	4.81	6.30
Glycine	4.48	6.40	5.05	5.76	5.58	5.94	5.39
Alanine	4.58	6.90	6.12	6.50	7.28	6.88	6.50
Valine	4.80	6.90	7.17	5.84	5.47	5.83	5.83
Methionine	1.37	1.10	1.99	2.06	1.86	2.16	1.47
Isoleucine	4.90	5.50	6.74	4.92	4.51	4.64	4.89
Leucine	7.98	10.00	9.28	9.20	8.35	8.89	8.68
Tyrosine	3.94	3.60	3.32	4.67	2.59	3.53	3.55
Phenylalanine	5.37	6.40	5.60	6.10	5.13	5.69	5.70
Tryptophan	-	1.20	1.52	1.50	2.36	2.10	1.04
Cysteine	-	1.50	1.05	1.25	1.30	1.70	1.37

*Grown in domestic sewage.

**Grown in artesion well water from NSTL wells.

***Grown in juice pressed from whole water hyacinth plants.

Table 7. Mineral Composition of Dry Duckweeds, Cattails, Water Hyacinth Leaves, Protein Extract From Water Hyacinth Leaves, Cattail Tops and Yeast Cells, PPM.

Material	Ca	K	Na	Zn	Fe	Cu	P	S
Duckweed no. 1	13400	12200		28.1	143	3.07	3640	4500
Duckweed no. 2	8770	21300	7360	1885	1450		10100	8500
Water hyacinth leaves	7560	36000	18300	23.0	143	8.0	9270	4500
Leaf protein extract	16500	4210	2660	21.3	146	19.5	4000	-
Yeast cells	220	251	9910	77.8	40.1	-	7280	-
Cattails	13700	19700	21500	35.4	94.6	13.0	3960	1090

Table 8. Vitamin Content of Dry Duckweeds, Water Hyacinth Leaves, Protein Extract From Water Hyacinth Leaves and Cattail Tops.

Material	Ribo-flavin PPM	Thiamine HCl PPM	Vit. B-12 PPM	Pyroxi-dine HCl PPM	Niacin Bound PPM	Vit. C PPM	Vit. E I.UM	Pantothenic Acid PPM
Duckweed no. 1	18.8	6.64	0.26	8.76	6.87	578	*	27.9
Duckweed no. 2	26.4	13.3	0.56	8.82	130	49.0	10.3	55.0
Water hyacinth leaves	30.0	5.91	.03	15.2	79.4	*	30	55.6
Leaf protein extract	8.42	4.77	*	0.61	2.8	13.0	14	10.1
Cattails	27.5	0.60	.03	4.55	43.4	32.0	*	1.86

*No data

V. Decomposing Water Hyacinths and Duckweeds As a Substrate for Growing Food Plants

The ability of water hyacinth plants to act as a complete growth media while being biologically decomposed was demonstrated by growing grain sorghum (Sorghum sudanese), corn (Zea mays), potatoes (Solanum tuberosum), cucumbers (Cucumis sativus), yellow squash (Cucurbita pepo), and cherry tomatoes (Lycopersicon esulentum) in a substrate of decomposing water hyacinths.

Cherry tomatoes were germinated in potting soil and four week old plants were transplanted into decomposing water hyacinth plants that had been grown in domestic sewage (Figure 4). Two hundred and forty-four grams (244 g) of dried water hyacinths were chopped and placed in 25 cm plastic pots which were placed in holes cut out of a 25 cm diameter plastic pipe. A distilled water reservoir, containing 2 liters of water per plant pot, was placed at the lower end of the plastic drain pipe. Distilled water was pumped through each pot for 15 minutes every hour on a 24-hour cycle. This schedule was changed 30 days later to 15 minutes every 6 hours because of excess water in the compost. After growing approximately 5 months in decomposing water hyacinths, each plant had produced an average of 100 tomatoes per vine with a total height of approximately 3.4 m (11 ft.). Fifty grams of dry water hyacinths were decomposed per pot during the first 3 months of operations and 150 g per pot during the last two months. The experiment was terminated after 5 months because of a leaf insect infestation. The decomposing water hyacinth liquid reservoir contained the following averages: total dissolved solids, 2500 mg/l; total kjeldahl nitrogen, 20 mg/l; total phosphorus, 20 mg/l; and a pH average of 8.2 during the 5 month experimental period. The decomposition of dried water hyacinths during the last 2 months was at a rate equal to that which plants can be grown under optimum growth conditions. Grain sorghum, corn, cucumbers and yellow squash were grown in 124 cm square greenhouse plant growth troughs (Figure 5). These troughs were filled to a depth of 28 cm with water hyacinths that were harvested from domestic sewage lagoons and allowed to dewater several weeks before use.

Figure 4. Hydroponic System for Growing Cherry Tomatoes in Decomposing Water Hyacinths, Raw Sewage and Dilute Urine Solutions.

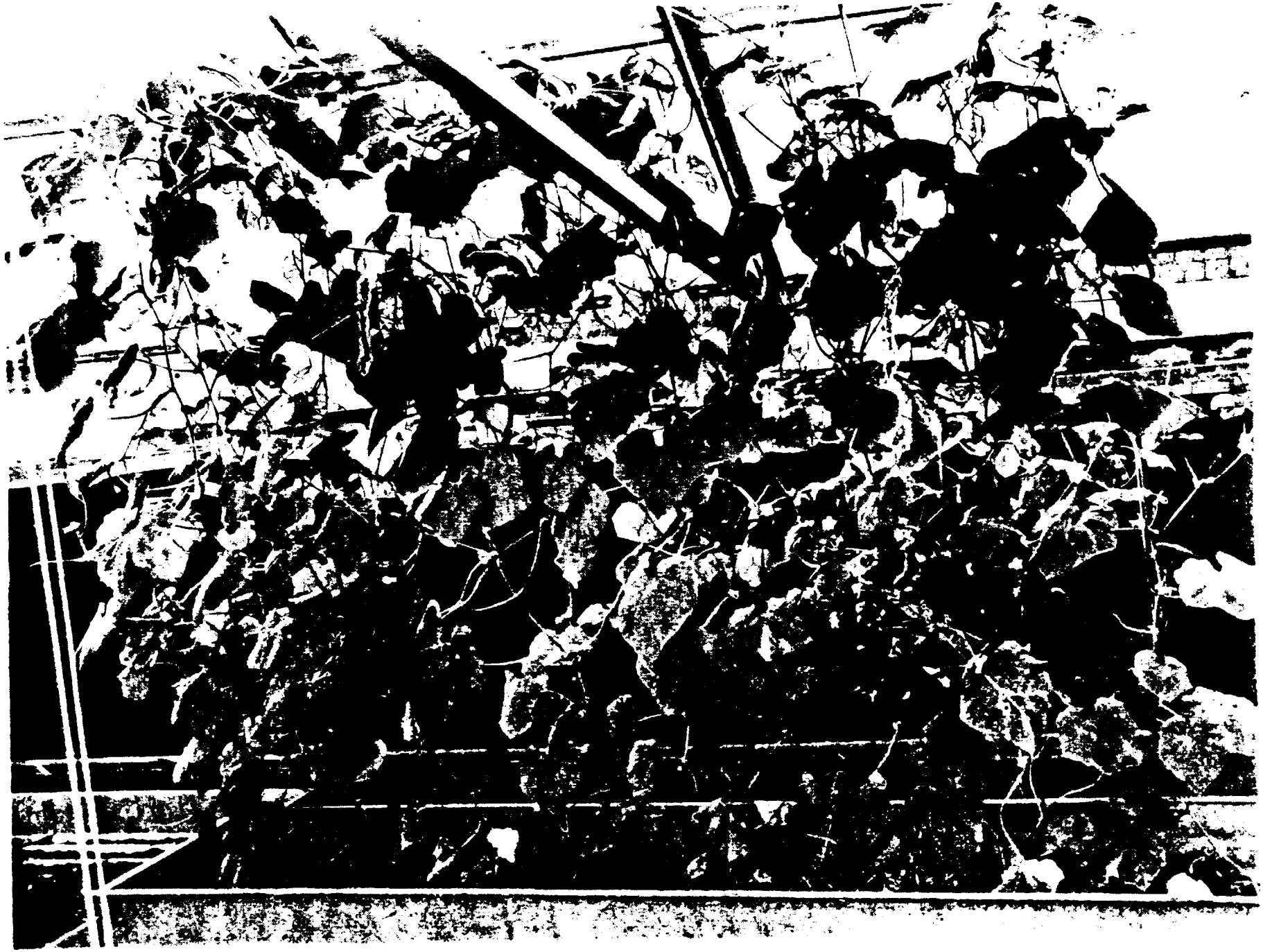


Figure 5. Cucumbers Growing in Decomposing Water Hyacinths.

Plants were hand watered when the top surface of the decomposing water hyacinths became dry with no additional plant material added to the troughs. The breakdown and release of nutrients from the decomposing water hyacinths were balanced with the growth rates of all plants studied except potatoes. Advanced decomposition before planting or the addition of duckweeds is desirable for potatoes because of the early demands of available nutrients during formation of the potato tubers. Potatoes were successfully grown in 40 cm diameter by 40 cm deep metal containers with a 10:1 dry weight ratio of water hyacinths to duckweeds. Each container contained an initial volume of 1.65-kg dry water hyacinths and 165 g dry duckweeds. After the potatoes were 1 month old an additional 2.00 kg of dried water hyacinths and 200 g of dried duckweeds were added to each container. After approximately 65 days the potatoes were harvested. An average of 12 potatoes were harvested from each vine with an average weight of 75 g each. Tomatoes, cucumbers, squash and potatoes produced from these experiments were consumed by Laboratory Personnel over a 2 year study period.

VI. Cherry Tomatoes Grown in 0.5% Urine Solutions

Cherry tomatoes (Sugar Lump) were germinated in potting soil and 3 week old plants were transplanted into vermiculite filled plastic pots (20 cm) and grown under hydroponic conditions. Tap water containing 0.5% urine was pumped through the tomato roots for 15 minutes every hour on a 24-hour cycle. From 600 to 1000 ml of solution was supplied/plant/pump cycle and approximately 10 liters of urine solution per plant were renewed every seven days. The experiment was initiated in October and terminated in March. Plants were grown under greenhouse conditions and were producing new tomatoes when terminated. After growing approximately 4 months in a 0.5% urine solution, the cherry tomatoes had produced an average of 270 tomatoes per vine with a total height of approximately 3.4 m (11 ft.). The average growth rate per day was 2.5 cm (1 in.). The harvested plant biomass averaged 1000 g/vine containing approximately 13% dry solids. The dried plants contained 12.2% ash, 0.965% calcium, 1.68% chloride (soluble), 1.82% sodium, 2.42% potassium, 1.99% nitrogen, 1.371% magnesium and 40.6% carbon. The average fresh weight of the ripe tomatoes was 16 g and contained 67 mg of sodium chloride per tomato compared to only half this concentration for tomatoes grown commercially. Daytime greenhouse temperatures averaged 29°C ± 3 with nighttime temperatures averaging 18°C. The average mineral concentrations in the urine solutions were: total kjeldahl nitrogen, 55 mg/l; total phosphorus, 11 mg/l; calcium, 2.3 mg/l; sodium, 115 mg/l; potassium, 14 mg/l; magnesium, 0.66 mg/l; and total dissolved solids 365 mg/l.

VII. Air Revitalization

It is a well-known fact that the uptake of carbon dioxide and production of oxygen by plants as well as the release of water vapor during transpiration is accomplished by the formation of new plant biomass. Approximately one liter of oxygen is liberated for each gram (dry) of new plant material produced during photosynthesis with equal or slightly less quantities of carbon dioxide being removed. The ability of higher plants to remove toxic impurities from both water and the atmosphere has also been demonstrated (8, 10, 11, 12, 13).

Vascular aquatic plants such as water hyacinths can maintain a dynamic growth rate through continuous production of new plants allowing for optimum oxygen production and CO_2 removal. With a growth rate of over 10% daily demonstrated at NSTL, less than a $9m^2$ surface area of water hyacinths should be able to supply the daily oxygen requirements (650 liters) per person/day. Vegetables growing on decomposing plant material also produce oxygen at a significant rate and will be an integral part of the carbon dioxide removal, oxygen production cycle.

VIII. Single Cell Protein From Water Hyacinth Juice

Juice from water hyacinths can also serve as a complete substrate for growing single cell protein. Approximately 650 ml of plant juice can be produced per kg of freshly harvested water hyacinths when processed through a screw press. Juice prepared by homogenation with distilled water and heated for 30 minutes at 110°C supports the growth of Candida tropicalis. An equal amount of dry yield yeast biomass can be recovered from plant juice and Standard Sabouraud Dextrose Broth, a highly refined nutrient commonly used for the propagation of yeast (34). The nutritional composition of the single cell protein produced under these conditions is shown in Tables 5, 6, and 7.

IX. The Removal of Biochemical Oxygen Demanding Substances (Organics) by Higher Plants

The ability of higher plants to absorb and metabolize organic chemicals is the basis for a systemic insecticide industry, but some scientists are still under the opinion that the absorption and metabolic degradation of organics are reserved for microorganisms alone.

Laboratory studies were conducted at NSTL under wide spectrum growth lights with 14 hour photoperiods in an effort to obtain more exact data on the ability of

water hyacinths to remove biochemical oxygen demanding (BOD) substance from water systems. Phenol, an organic chemical, was also used in these studies to further demonstrate the ability of water hyacinths to absorb, metabolize and remove BOD in a similar manner to microorganisms (35). Domestic wastewater consists of a complex mixture of chemicals including phenol and related organics. The initial volumes of raw sewage or phenol solutions were varied in order to vary the depth and surface to volume ratio. Some containers were left free of water hyacinths as controls to determine the bacterial contribution to BOD removal. In order to assure the same type of bacteria would be present in the controls that were associated with the water hyacinth roots, the plant roots were first dipped in all control solutions for bacterial seeding.

Results of these experiments are shown in Tables 9, 10 and 11. This data indicates that the water hyacinth alone can be expected to reduce BOD_5 of domestic sewage by an average of 1.5 mg BOD_5 per gram of plant mass (wet plant weight) with liquid detention times of 6 to 7 days. These values are consistent with those found with domestic sewage.

These BOD removal rates were achieved with daily growth rates of only 3-4%; whereas, field studies have shown daily growth rates as high as 8% when grown in sewage lagoons in south Mississippi, with greenhouse growth rate of over 10% being demonstrated. The BOD removal rate does not appear to be entirely dependent on growth and harvesting rates; whereas, the removal of nutrients such as nitrogen, potassium and phosphorus and the production of oxygen is dependent on these variables. The BOD removal rate is dependent on root absorption and metabolic functions.

Table 9. 5-day Biochemical Oxygen Demand (BOD_5) and Bacteria Concentrations in Raw Sewage With and Without (Control) Water Hyacinths.

Experiment	Fresh Mass WHs, g	Total BOD_5 mg/l			mg BOD_5 removed/ 6 Days	mg BOD_5 removed/ g WHs (6 day exposure)	Bacteria, Count/100 ml		
		Initial	3rd Day	6th Day			Initial	3rd Day	6th Day
1. w/WHs	1,860	60	---	5	4,070	2.2	8.0×10^5	---	3.0×10^4
2. Control	0	60	---	24	2,664	---	8.0×10^5	---	3.1×10^4
3. Control	0	60	---	35	1,850	---	8.0×10^5	---	2.3×10^4
4. w/WHs	2,140	180	48	9	12,664	5.9	7.7×10^5	1.0×10^4	1.0×10^4
5. w/WHs	2,000	180	36	7	12,802	6.4	7.7×10^5	6.5×10^4	5.0×10^3
6. Control	0	180	100	65	8,510	---	7.7×10^5	3.6×10^4	1.4×10^4

Conditions: Mean Atmospheric Temperature: 22°C

Volume of Raw Sewage: 74 l

Depth: 61 cm

Table 10. 5-day Biochemical Oxygen Demand (BOD_5) And Bacteria Concentrations in Raw Sewage With and Without (Control) Water Hyacinths.

Experiment	Fresh Mass WHs, g	Total BOD_5 mg/l			mg BOD_5 removed/ 7 Days	mg BOD_5 removed g WHs (7 day exposure)	Bacteria, Count/100 ml		
		Initial	4th Day	7th Day			Initial	4th Day	7th Day
1. w/WHs	506	190	36	20	2040	4.0	TNTC	1.0×10^5	38×10^5
2. w/WHs	429	190	40	20	2040	4.7	TNTC	3.0×10^5	231×10^5
3. w/WHs	413	190	38	21	2030	4.8	TNTC	1.0×10^5	208×10^5
4. Control	0	190	170	85	1260	---	TNTC	1.0×10^5	44×10^5
5. w/WHs	376	112	*50	**21	**1090	**2.9	7.0×10^6	*3.3×10^6	**2.5×10^6
6. w/WHs	412	112	46	18	1130	2.7	7.0×10^6	4.3×10^6	2.7×10^4
7. w/WHs	386	112	42	22	1080	2.8	7.0×10^6	2.7×10^6	1.2×10^5
8. Control	0	112	76	48	768	---	7.0×10^6	*** TNTC	4.5×10^5
9. Control	0	112	69	60	624	---	7.0×10^6	3.1×10^6	3.1×10^6
10. Control	0	112	60	48	768	---	7.0×10^6	2.2×10^6	3.6×10^5

* 3rd day for experiments 5-10

** 6th day for experiments 5-10

*** TNTC - Too numerous to count

Conditions: Mean Atmospheric Temperature: 29°C

Volume of Raw Sewage: 12 l

Depth: 15 cm

Table 11. 5-day Biochemical Oxygen Demand (BOD_5) and Bacteria Concentrations in 100 mg/l Phenol Solutions With and Without (Control) Water Hyacinths.

Experiment	Fresh Mass WHs, g	Total BOD_5, mg/l		mg BOD_5 removed/7 days	mg BOD_5 removed g WHs (7 day exposure)	Bacteria, Count/100 ml	
		Initial	7th Day			Initial	7th Day
1. Control	0	160	114	184	---	106×10^5	250×10^4
2. Control	0	160	120	160	---	148×10^5	51×10^4
3. Control	0	160	115	180	---	115×10^5	174×10^4
4. w/WHs	155	160	35	500	3.2	110×10^5	61×10^4
5. w/WHs	200	160	37	492	2.5	37×10^5	82×10^4
6. w/WHs	298	160	35	500	1.8	143×10^5	24×10^4
7. Control	0	235	136	396	---	3×10^4	34×10^5
8. Control	0	235	115	480	---	1×10^4	TNTC
9. Control	0	235	116	476	---	2×10^4	---
10. w/WHs	120	235	26	836	7.0	1×10^4	60×10^6
11. w/WHs	242	235	15	880	3.6	1×10^4	3×10^5
12. w/WHs	293	235	29	824	2.8	1×10^4	TNTC

Conditions: Mean Atmospheric Temperature: 29°C

Volume of Phenol Solution: 4 l

Depth: 13 cm

CONCLUSIONS

A promising scheme for constructing a versatile closed bioregenerating life support system using higher plants has been formulated. Six years of extensive research with water hyacinth for wastewater treatment and biomass production has been conducted at NSTL which demonstrates the potential of this plant in waste recycling and food production in closed systems. Two years of limited research with duckweed, cattail, tomatoes, and conventional food plants have also demonstrated the potential of these plants in balancing and stabilizing a bioregenerative life support system. This research demonstrates that: (a) higher plants such as water hyacinth, duckweed, cattail and cherry tomatoes can utilize human waste as a complete growth media, (b) conventional food plants such as tomatoes, sorghum, corn, potatoes, cucumbers and squash can utilize decomposing water hyacinth and duckweed as a substrate for supplying all of their nutritional growth requirements, (c) the biological decomposition rate of water hyacinths approaches or equals its growth rate when the harvested plants are used as a substrate for growing other plants, (d) water hyacinth and duckweed have the potential of being used directly or indirectly for supplying man's food requirements, (e) juices from water hyacinth can be used to produce high quality protein in the form of yeast cells, (f) water hyacinth can absorb and metabolize organics directly from wastewaters acting in a chemical filtration capacity, and (g) a combination of water hyacinth, duckweed and cattail is capable of maintaining growth under varied light and temperature fluctuations.

A life support phytotron system consisting of two separated modules containing C_3 and C_4 plants shows promise of adding flexibility to this system. C_4 plants such as sorghum and corn can utilize carbon dioxide at very low levels and photosynthesize in a high oxygen environment. The opposite characteristics are applicable to C_3 plants.

A phytotron system similar to the one discussed in this paper should be constructed, and the tedious task of material balance using input waste from 1-3 people started. The first mass balance studies should be directed toward nutrient and water recycling from waste and dehumidification, respectively, because a less than absolute sealed system can still produce valuable information in this area as well as in the biological recycling of inedible plant parts.

Future experiments should include gas exchange studies in completely sealed phytotron units on earth with the most promising plant candidates subjected to an actual deep space environment to determine radiation and gravity effects.

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